Peterson's Press

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UP FRONT

The Role of Anti-CCP in the Laboratory Diagnosis of Rheumatoid Arthritis

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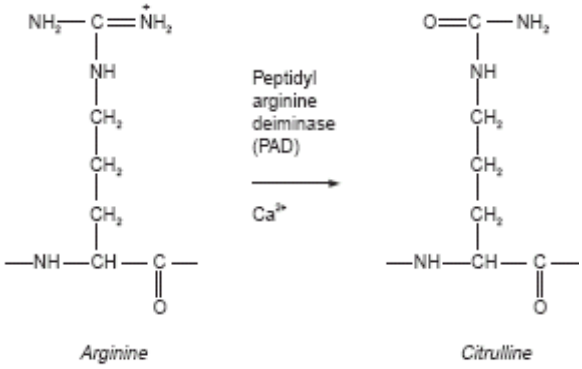
CAP Diagnostic Immunology Resource Committee

Rheumatoid Arthritis (RA) is one of the most common systemic autoimmune diseases, affecting approximately 0.5–1.0% of the world population. The American Rheumatism Association criteria for the classification of RA includes: 1) morning stiffness, 2) arthritis of 3 or more joint areas, 3) arthritis of hand joints, 4) symmetric arthritis, 5) rheumatoid nodules, 6) serum rheumatoid factor (RF), and 7) radiographic changes. A patient should have four of the seven criteria to be diagnosed with RA and the first four criteria should be present for at least six weeks. Until recently, the only serological test routinely performed for the detection of RA was for the presence of IgM RF. RF is found in approximately 50%–90% of these patients, but it is also found in patients with infections, other autoimmune diseases, and some healthy individuals with increasing frequency in older age groups, thus limiting its specificity for RA.

Several studies have shown that anti-perinuclear autoantibodies, otherwise known as anti-keratin autoantibodies, are found in patients with RA. It has been discovered that these antibodies recognize an epitope that contains the deamidated form of arginine called citrulline. Enzyme-Linked Immunosorbent Assay (ELISA) testing for these autoantibodies directed against anti-cyclic citrullinated peptide (anti-CCP) is reasonably sensitive (68%) and highly specific (98%) in patients with RA. The pathogenesis of anti-CCP antibodies in rheumatoid arthritis has been shown to be attributable to the body's humoral response to citrulline. Citrullination is the post-translational conversion of arginine to citrulline by an enzyme called peptidylarginine deiminase (PAD). See figure 1. PAD activation is assisted by calcium ions. PAD is normally present as inactive intracellular enzymes. During programmed cell death (apoptosis) in the synovial joints of patients with rheumatoid arthritis, PAD may leak out of the dying cells. Once activated, PAD will cause citrullination of extracellular arginine. In the synovium, the citrulline acts as an antigenic stimulant to induce anticitrullinated protein antibodies (ACPA) locally produced by plasma cells. The ELISA that detects these autoantibodies uses synthetic cyclical citrulline peptides.

Figure 1



The enzymatic conversion of protein-contained arginine to citrulline.

The original ELISA for the anti-CCP sequence was not broadly marketed due to low sensitivity and technical complexity. However, the second generation anti-CCP test (often referred to in the literature as CCP-2/CCP2) shows superior performance compared to the original peptide. The vast majority of the labo-

ratories that offer this test utilize the second-generation CCP assay.

In 2005, a third generation of anti-cyclic citrullinated peptide (CCP3) was made available for the laboratory diagnosis of RA. These assays have been reported to recognize additional citrulline epitopes that are not identifiable with the second-generation CCP assays. The CCP3 assays have had reported results of up to 5% increased sensitivity compared to the CCP2 assays. To the contrary, however, several publications have shown similar diagnostic performance between the CCP3 and CCP2 assays.

Recently, Nishimura et al. performed a meta-analysis of published studies regarding the diagnostic accuracy of anti-CCP and RF for rheumatoid arthritis. Their results showed a positive likelihood ratio of 12.46 and a negative likelihood ratio of 0.36 for anti-CCP antibody in patients with RA. The same study showed a positive likelihood ratio of 4.86 and a negative likelihood ratio of 0.38 for RF. These results indicate that anti-CCP positivity alone is more specific than IgM RF for the diagnosis of RA.

In addition to diagnostic value, several studies have shown that anti-CCP may also add prognostic significance in the determination of development of erosive disease in RA. Kroot, EJ et al showed that anti-CCP positive patients developed significantly more severe radiologic damage than those patients who were anti-CCP negative.

Although the presence of anti-CCP is not currently required for the diagnosis of RA, future classification criteria will most likely incorporate its use as an adjunct to IgM RF as a laboratory diagnostic tool. Additionally, RA patients with positive CCP status may benefit from its prognostic value by receiving earlier customized treatment regimens that could potentially delay the development of erosive disease.

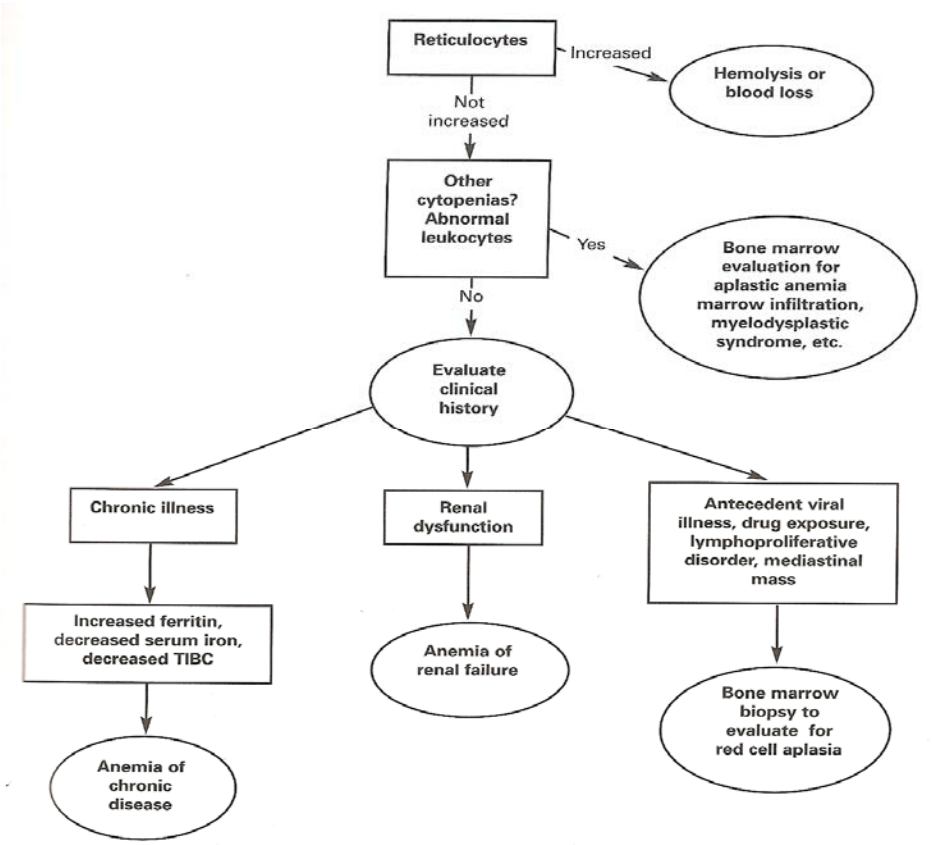
Laboratory Evaluation of Normocytic Anemia

Alexandra Harrington, MD
CAP Hematology and Clinical Microscopy Resource Committee

Anemias can be classified according to the mean corpuscular volume (MCV) into microcytic, normocytic and macrocytic. In normocytic anemias, the MCV is within the reference range, generally between 80-100 fL. Though anemia of chronic disease (ACD) is the most common cause of such anemias, the differential diagnosis is extensive, including blood loss, hemolytic anemias, anemia of renal disease, nutritional anemias and primary bone marrow disorders. Multiple diagnostic algorithms are available; a concise and clinically useful example is shown in the figure below.

Normocytic anemias can be initially categorized based on the reticulocyte count, into those characterized by effective erythropoiesis (elevated reticulocytes) or ineffective erythropoiesis (low to normal reticulocytes). Hemolytic anemias and anemias secondary to blood loss will have elevated reticulocyte counts in the days following episodes, as red cell production is not impaired. In contrast, nutritional anemias, ACD, anemia of renal disease and the anemias secondary to primary bone marrow disorders are examples of disorders of diminished red cell production and, therefore, have low to inappropriately normal reticulocyte counts.

Examination of the peripheral blood smear is as essential as reticulocyte enumeration in the initial work-up of a normocytic anemia. Attention must be paid to all three cell lineages, as abnormalities in each may provide diagnostic clues. For example, many of the hemolytic anemias have characteristic peripheral blood features, such as sickle



cells in sickle cell disease, spherocytes in hereditary spherocytosis (HS) and autoimmune hemolytic anemia (AIHA), schistocytes in microangiopathic hemolytic anemia (MAHA) and bite cells in glucose-6-phosphate dehydrogenase deficiency. Cytopenias, circulating immature precursors, including a leukoerythroblastic reaction, and dysplastic features suggest underlying bone marrow disorders and require bone marrow examination.

If hemolysis is suspected and supported by decreased haptoglobin with elevated indirect bilirubin and lactate dehydrogenase levels, further evaluation is needed. Because the hemolytic anemias encompass a wide variety of disorders—including AIHAs, MAHAs, hereditary and acquired red cell membrane defects, such as HS and paroxysmal nocturnal hemoglobinuria and hemoglobinopathies—additional work-up is tailored based on clinical suspicion and peripheral blood findings. For example, a direct antiglobulin test (Coombs test), which assesses immunoglobulin and/or complement bound to red cells, is requested to rule out an AIHA in a patient with spherocytes.

For normocytic anemias with decreased reticulocyte counts and fairly unremarkable blood smears, the differential diagnosis includes ACD, anemia of renal disease, nutritional anemias and red cell aplasia. In these cases, the clinical history may guide further laboratory evaluation, if needed. For example, in patients with chronic illness, iron studies are obtained to evaluate for ACD, with elevated serum ferritin levels, decreased serum iron levels and decreased total iron-binding capacity (TIBC) confirming the diagnosis. Likewise, in patients with poor diets, it may be appropriate to assess iron status and/or vitamin B_{12} and folate levels, as deficiencies of these nutrients may rarely present as normocytic anemias. Finally, bone marrow examination is required for evaluating the etiology of red cell aplasia.

In summary, the differential diagnosis of normocytic anemia is vast. Evaluation begins with reticulocyte enumeration and blood smear examination, with further work-up based on these findings and clinical history.

CMS Centers for Medicare & Medicaid Services

MEDICARE TIP OF THE WEEK

Are you unsure if a Local Coverage Determination policy exists for a procedure you bill?

CMS offers a search tool that can locate and identify if an LCD exists. All you need to do is enter a keyword and the geographic area for the policy. A keyword can be a CPT code, a narrative descriptor, a Dx code or other information you may be seeking.

To access the LCD search on the CMS website, refer to the following address, choose "Local Coverage Documents," enter the keyword and geographic area, and click, "Search Now," to search only the title of LCDs. To search LCD documents in their entirety, you will need to click on "Advanced Search."
www.cms.hhs.gov/mcd/search.asp?from2=search.asp&

OUR JOURNEY TOWARD GREEN

We have recycled our alcohol, xylene and phone books for several years. Recently, we began recycling the plastic, aluminum, paper and cardboard we generate.

Laboratory Recycling Area

Five feet of available floor space was dedicated to our "recycling area." Lab couriers deliver the collected materials to the local recycling center as the containers fill.

In addition, we are vigilant about the paper we generate.

Did you know:

- Office paper is highly recyclable
- The average office worker uses **10,000 sheets** of copy paper each year — totaled with other office workers, it is enough to build a 12' wall from New York to San Francisco
- It takes more than 1½ cups of water to make one sheet of paper (picture a typical soda can).
- Reducing paper use reduces greenhouse gases: comparable to 3/4 acre of pine forest absorbing carbon for every office worker.
- The costs of <u>using</u> paper in the office can run 13 to 31 times the cost of <u>purchasing</u> the paper in the first place!

WEBSITE TRAINING RESOURCES

Like most of your facilities, our website has grown and developed since its inception five years ago.

- Visitors "hit" our website nearly 60,000 times last year, with 18,000 page visits
- We have seen particular interest in the area of archived training resources
- Nearly 80% of our visitors connect through a bookmark
- 42% of visitors add us to their favorites
- There are 113 total pages
- Among the most popular training resources recently are the Lab Safety audio-conference series, CLSI anti-microbial susceptibility standards handouts, and "Phlebotomy Made Simple"

We are again sponsoring the 2009 CLSI Anti-Microbial Susceptibility Testing Standards Teleconference in January. Watch for that archive to be available after February 1. To access the training opportunities page, go to

www.petersonlab.com/about/inservice.html

IN THE SPOTLIGHT: HISTOLOGY DEPARTMENT

L to R: Travis Troyer, HT(ASCP), Tonya Daws, HT(ASCP), Michael Avery, D-ABMDI, and Kay Poindexter, HT(ASCP)

HISTOLOGY DEPARTMENT

We are proud to introduce to you the skilled and friendly team who process and report more than 14,000 of your surgical specimens each year.

Michael Avery, D-ABMDI, HistologySupervisor/Pathology Assistant: Mike joins Dr. Speaks, Dr. Salem and Maureen Jensen in the ranks of Nebraska-raised Cornhusker fans. Originally from Lincoln, Nebraska, Mike attended Southeast Community College after graduating from Lincoln Northeast High. Prior to lending his experience and talents to Peterson Laboratory Services in 2001, Mike was a pathology assistant at Pathology Medical Services in Lincoln. As a Diplomat, American Board of Medicolegal Death Investigators, Mike responds to coroner requests in the absence of the district coroner (Dr. Bambara).

Mike finds the variety of his work responsibilities and performing gross examination of surgical specimens among the most rewarding aspects of his position. Guess what he reads for enjoyment —non-fiction crime novels! His son, Cory, is following in his footsteps as a pathology assistant in Lincoln, NE. Mike and his wife, Brenda, are the parents of four children and live in Blue Rapids.

Kay Poindexter, HT(ASCP), Histotechnician: Kay carries the distinction of being the "senior tenure" team member — devoting her skills to preparing specimens for pathology review since 1998. Kay is a true Manhattan-ite, born and raised. After high school graduation, she earned her medical assistant degree from Brown Mackie College in Salina. Kay has extensive previous medical and nursing experience. Both Kay and Travis begin processing specimens at 5:30 each morning, and she identifies the early shift and her role in patient diagnosis was gratifying.

"Peterson Laboratory does a truly outstanding job of customizing their services to meet client needs. As an example, they provide quarterly statistics to assist us in meeting the State Tumor Registry reporting requirements.

Online reporting? I love it! Everyone can use it—nurses for tracking patient care, front office for chart prep, and billing staff for obtaining diagnosis codes as quickly as possible."

Rosanna Mitchell, Administrator Associated Urology, PA

In her off-work hours, Kay stays busy riding her Yamaha V-Star motorcycle, exploring Kansas and participating in poker runs with her husband. Lawrence is among their favorite locations to tour. Accomplished at crochet, Kay is currently teaching herself to knit.

Travis Troyer, HT(ASCP), Histotechnician: Travis spent the first four years of his life in Pawnee City, Nebraska. He is a graduate of Riley County High School and holds a microbiology degree from Kansas State University. Travis dedicated his early career to Food Labs in Manhattan, where meat was tested for bacterial contamination. He has been an employee of Peterson Laboratory Services since 2000. He finds great work fulfillment in performing immuno and other special stains. " They (the stains) can make all the difference in obtaining a definitive specimen diagnosis, " he states. Travis is an entertaining father to his daughter (age 7) and son (age 4). He and his wife reside in Manhattan. If you're lucky, you catch Travis performing with the Little Apple Barbershop Chorus — including "Singing Valentines!"

Tonya Daws, HT(ASCP), Pathology Assistant: Tonya was born and raised in Salina, KS; graduating from Salina South High School. Her parents and brother are Salina residents. Tonya earned a biology degree from Kansas State University. Her previous work experience includes seven years at Salina Regional Health Center. Prior to her role as a pathology assistant, Tonya began her career at Peterson Laboratory Services as a courier and then a non-genital cytology technician. With true youthful enthusiasm, Tonya enjoys "everything!" about her work, especially the discovery of pathological changes and the variety of surgical specimens in gross examination. Hats off to Tonya for having the patience and fortitude to raise her two purebred Cocker Spaniels (Ranger and Zorro) from puppy-hood.

SERVICE IS OUR PRIORITY:

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